

The effect of combinations of sourdough lactic acid bacteria and yeasts on wheat bread quality: Evaluation in terms of microbiological, rheological, textural and volatile components

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ABSTRACT In this study, the effect of co-cultivation of lactic acid bacteria (*Lactiplantibacillus plantarum* 45MK-30, *Limosilactibacillus fermentum* 29GT-19) and yeasts (*Saccharomyces cerevisiae* 06B-39, *Kazachstania humilis* KCO-60 and *Pichia membranifaciens* 11G-11) on the quality of sourdough bread was studied. Two different fermentation phases were used (I and II) and bread making was carried out in the II phase of fermentation. Microbiological analysis, pH and TTA values were checked in these two phases. While cell density and pH value of microorganisms were close to each other in I phase fermentation, a decrease in these values was observed in II phase fermentation. When the rheological analyzes of the doughs at the end of phase II are examined, all doughs exhibited lower elastic behavior. It can be said that the textural feature of sourdough breads is softer compared to both controlled acidified (CA) bread and non-sourdough (flat dough, CY) bread. Another parameter examined in breads was the evaluation of volatile components by HS-GC / MS. It was observed that breads produced by co-cultivation had more volatile components than plain dough bread.

KEYWORDS LAB; Yeast; Microbial interactions; Bread

1. Introduction

Sourdough is a product formed by the mixture of flour and water and fermented by lactic acid bacteria (LAB) and yeasts. As a result of the mixture of microorganisms in the sourdough culture, starch and sugars in the flour are fermented to form CO₂. This leads to the production of aerated bread (Arendt et al., 2007; De Vuyst et al., 2009; Winters et al., 2019). The microbial community consisting of LAB and yeast is responsible for this fermentation process (Vogel et al., 1999; Carbonetto et al., 2020). Microbial diversity occurs in sourdough with the dominance of LAB and yeast in sourdough (Akinola and Osundahunsi, 2017; Akinola et al., 2020). It has been stated that sourdough contains mainly organic acid producing LAB and yeasts producing CO₂ and ethanol as by-products of metabolism. Interactions between yeasts and LAB are important for the metabolic activity of sourdough (Poutanen et al., 2009; Akinola et al., 2020). Generally, LAB produces metabolites for sourdough during fermentation. Thanks to these metabolites produced, it contributes to the quality of sourdough baked foods (Oshiro et al., 2021). In addition to these properties, the metabolic activities of LABs cause changes in gluten structure and affect the texture of cooked foods as they produce acid. This acid is produced during a change in the structure of gluten which is a protein complex (Corsetti and Settanni, 2007; Koehler and Wieser, 2013; Oshiro et al., 2021). Acetic

acid shortens the gluten structure and makes it harder. Lactic acid gradually creates a more elastic gluten structure (Corsetti and Settanni, 2007; Oshiro et al., 2021). In addition, sourdough LABs are among the properties known to contribute to the flavor and shelf life of the final product (Gänzle and Ripari, 2016; Oshiro et al., 2021). Another point has been that some yeast strains produce more flavor metabolites in the presence of LAB (Maloney and Foy, 2003). Bringing the benefits of sourdough fermentation and microbial diversity to traditional bread production can play a role in making the bread healthier (Fernández-Peláez et al., 2020). Therefore, it is important to understand the consequences of yeast and bacterial strains. Also, their interactions within the co-culture must occur during the fermentation stage (Winters et al., 2019). The coexistence and interactions of yeast and LABs affect ecosystem function and permanence. Understanding how bacteria and yeast interact sheds light on both the ecology and mechanisms of their interactions. It also enables the development of new microbial starters for the food industry (Carbonetto et al., 2020). The use of LAB and yeasts in sourdough not only improves processing conditions and product quality, but also provides accelerated acidification, improved and more predictable fermentation process, desirable sensory properties, and improved safety and reduction of hygienic risks (Holzapfel, 2002; Arendt et al., 2007; Sieuwerts et al., 2008; Olojede et al., 2020).

Today, sourdough is produced under controlled conditions with selected LABs and yeasts (Sakandar et al., 2019). It is known that the use of sourdough improves the organoleptic quality. However, in some studies, it has been stated that adding sourdough to bread formulation has a positive or negative effect on flavor. In addition, it was emphasized that volatile components caused by

microbial activity in the fermentation process play a role from the aroma in sourdough bread (Farahmand et al., 2021).

Most previous sourdough fermentation studies concerned with yeast species *Saccharomyces cerevisiae* and there are no studies on the use of the combinations we used in making sourdough. For this purpose, LAB and yeasts isolated from sourdough were used as co-cultures and the effects of the combination on dough and wheat bread making were observed and the effects of different phases of fermentation were examined. By evaluating the effects of the resulting combination on bread quality, it was aimed to lead both future studies and the bakery industry. For this, the properties of sourdough bread prepared by co-culture were assessed by texture profile analysis (TPA). In addition, volatile compounds involved in the determination of the sensory properties of sourdough breads using gas chromatography GC / MS has been examined. In addition, the rheological properties of sourdough were another parameter determined.

2. Methods

2.1 Growth conditions of microorganisms

In this study, three yeast (*Saccharomyces cerevisiae* 06B-39, *Kazachstani humilis* KCO-60 *Pichia membranifaciens* 11G-11) and two lactic acid bacteria (*Lactiplantibacillus plantarum* 45MK-30 and *Limosilactibacillus fermentum* 29GT-19) isolated from conventional sourdough in Turkey were used in combination. Lactic acid bacteria strains were allowed to grow anaerobically in De Man, Rogosa and Sharp medium (MRS) at 37 °C for 24 h, and yeast cultures were allowed to grow aerobically in Yeast Peptone Dextrose medium (YPD) at 24 h 30 °C incubation.

2.2 Preparation of sourdough samples

Sourdough samples were prepared by making several modifications in the method specified by (Xu et al., 2019). Briefly, 10 gram of flour was weighed and 10 mL of microorganism culture (5 mL yeast + 5 mL LAB) was added and mixed and left to ferment at 30 °C for 24 h. Chemically acidified dough (acidified to pH 3.5 by acetic acid addition (0.27 % (v/w)) was fermented under the same fermentation conditions. Then, for the II phase of fermentation, 20 g of wheat flour, 20 g of sterile water and 10 g of sourdough (in the I phase of fermentation) were used and left to ferment again at 30 °C for 1.5 h. Sourdough prepared in combination and their codes are given in Table 1.

2.3 Making sourdough breads

For the making of sourdough breads after the 2nd fermentation, 70 g of wheat flour, 30 g of water, 1.85 g of salt, 0.5 g of commercial yeast, 60 g of II phase sourdough were used. For the comparison of breads, dough without sourdough was used as a control. The doughs were mixed for 8 minutes and left at room temperature for 15 minutes, then rested for 45 minutes, 25 °C by shaping. The oven temperature for bread was set at 190 °C, 30 minutes.

Table 1: Sourdough codes and combined starters.

Sourdough codes	Combined starters
A	<i>Saccharomyces cerevisiae</i> 06B-39 + <i>Lactiplantibacillus plantarum</i> 45MK-30
B	<i>Saccharomyces cerevisiae</i> 06B-39 + <i>Limosilactibacillus fermentum</i> 29GT-19
C	<i>Kazachstania humilis</i> KCO-60 + <i>Lactiplantibacillus plantarum</i> 45MK-30
D	<i>Pichia membranifaciens</i> 11G-11 + <i>Lactiplantibacillus plantarum</i> 45MK-30
E	<i>Pichia membranifaciens</i> 11G-11 + <i>Limosilactibacillus fermentum</i> 29GT-19
F	<i>Kazachstania humilis</i> KCO-60 + <i>Limosilactibacillus fermentum</i> 29GT-19

2.4 Determination of the cell density, pH and TTA (titratable acidity) values in sourdough

Microorganism count, pH and TTA values of sourdoughs were determined. For microorganism count, 5 g of sample was taken and 45 mL of sterile physiological saline (0.85 % v/v) was added. Subsequently, serial dilutions were prepared and inoculated on MRS and YPD agar with the spot method (20 µL for each spot). The MRS agar plate was incubated at 37 °C for 24 h, and the YPD agar plate at 30 °C for 48 h. Results are given as log CFU/g. In order to determine the pH and TTA values, 1 g of sample was weighed and homogenized with 18 mL of sterile distilled water and measured with a pH meter. For TTA, it was titrated with 0.1 N NaOH until pH 8.5.

2.5 Determination of viscoelastic properties of sourdoughs by rheometer

For the determination of viscoelastic properties, the sourdough co-cultured with starters were determined before making bread after 24 h fermentation. For the determination of rheological properties of dough, the method described by Alkay et al. (2022) was used. Briefly, Amplitude sweep test was applied in strain range of 0.1-100 Pa, 25 °C and 10 rad/s and determination of linear viscoelastic region. And then Frequency sweep test was performed from 10 to 100 rad/s at %0.5 strain and 25 °C. It is expressed as storage (G') and loss modulus (G'').

2.6 Texture profile analysis

After baking, the breads were left to rest for 1 hour and then TA.XTPlus model texture analyzer (Stable Micro System Co., Ltd., Surrey, England) device was used to determine the textural properties. The breads were cut as 2.5 cm and measured. Measurement parameters were used according to the method adapted by Alkay et al. (2020).

2.7 Profiles of volatile constituents in breads with HS-GC / MS

Gas chromatography-mass spectrometry was carried out using GCMS-QP2010 gas chromatography mass spectrometry system (Shimadzu, Milan, Italy) for determina-

tion of volatile components in breads. For this, 2 g of sample was transferred to 20 mL head space vials. For Headspace autosampler parameters, incubation temperature was set as 60 °C, incubation time as 7 minutes, agitation speed of 250 rpm, Helium flow rate as 1 mL/min. For the MS program, the injector temperature was used as 250 °C, mode 35–350 m / z range. In the GC-MS analyzes, A Restec (Bellefonte, USA) Rtx-5MS fused silica capillary column (30 m × 0.25 mm (internal diameter) 0.25 µm) was used for chromatographic separation.

2.8 Statistical Analysis

One-way analysis of variance (ANOVA) was used to determine the differences between co-cultured strains. JMP version 9 was used for this purpose. The calculation was given as mean ± standard deviation.

3. Results

3.1 Counting of cells, pH and TTA values

The amount of cells, pH and TTA values in the sourdoughs in the I and II phases of fermentation were given (Table 2). It can be said that the LAB and yeast counts of the sourdoughs in the I phase of fermentation are approximately the same between each other. LAB and yeast counts decreased in all doughs in II phase of fermentation. C and F coded sourdoughs had the same LAB count (8.61 CFU/g) in the I phase, while there was a significant decrease in the C coded sourdough (*Kazachstania humilis* KCO-60 + *Lpb. plantarum* 45MK-30) in the II phase. LAB and yeast are very important in food processing. During fermentation, LABs were in the predominant form and outnumbered yeast as they were responsible for acid production (Ewuoso et al., 2020). Another important parameter in our study is pH and TTA values. The pH values of the I stage of the sourdough fermentation were close to each other and ranged from 4.25 to 5.51. These values decreased in the II phases of fermentation and varied between 4.11 - 4.96. As the fermentation proceed, there was a decrease in pH values. It was observed that the sourdoughs had similar pH values. The acidity level of sourdough is important for the flavor and inhibitory activity of LAB against rop formation and other spoilage factors (Corsetti and Settanni, 2007). It was observed that the of TTA values increased as the pH value decreased. While the TTA values in the I stage were between 0.36 - 0.76, in the II stage these values changed between 0.38 - 0.81. The predominant metabolite in lactic acid fermentation is lactic acid, as well as acetic acid can contribute to acidification. In many other studies, there is a symbiotic relationship between yeast and LABs in fermented foods. While yeasts produce vitamins to support the development of LABs, LABs produce acids for the growth of yeasts (Odunfa and Adeyele, 1985; Ewuoso et al., 2020). When our results were evaluated in general, it was seen that the dough belonging to the yeast strain *Saccharomyces cerevisiae* (I phase) exhibited higher pH value. The lowest pH value in both phases was observed in *Pichia membranifaciens* yeast cultured dough. Karaman et al. (2018) created a combination between yeast and LAB in sourdoughs in their study. They also indicate that the highest pH drop

occurs in sourdough containing strains of *P. membranifaciens* + *Lb. brevis*. Whereas the sourdough belonging to the highest pH value is *S. cerevisiae* + *P. membranifaciens*. These parameters were parallel to our results.

3.2 Textural properties

In the study, properties such as hardness, springiness, cohesiveness, gumminess, chewiness and resilience of sourdough breads prepared with combinations of starters were examined (Table 3). It is seen that the hardness levels of sourdough breads are close. However, the E coded sourdough bread (*Pichia membranifaciens* 11G-11 + *Limosilactibacillus fermentum* 29GT-19) had the highest hardness. It is seen that there is a parallelism between gumminess and chewiness values. The sourdough bread of *Pichia membranifaciens* yeast strain had the highest chewiness values among sourdoughs. There was no significant difference in springiness among all sourdough breads associated with resilience (Carocho et al., 2020; Jin et al., 2021). Physicochemical changes such as acidification by LAB, microbial hydrolysis of starch and proteolysis effect in sourdough cause some changes in bread. This delay both bread hardness and staling (Corsetti et al., 2000).

Interactions between yeasts and lactobacilli are important for the metabolic activity of sourdoughs (Poutanen et al., 2009; Akinola et al., 2020). It is known that the production of organic acids (lactic, acetic), ethanol, the production of aroma compounds, bacteriocins, and metabolites such as exopolysaccharides by the LAB improves the texture by extending the shelf life of bread (Ewuoso et al., 2020).

3.3 Determination of volatile compounds by HS-GC / MS analysis

Sourdough fermentation can contribute to the flavor and aroma of baked bread (Kim et al., 2009; Wu et al., 2012; Yang et al., 2020). In our study, HS / GC-MS analysis was applied to evaluate the volatile compounds of sourdough bread prepared by co-culture sourdough and flat dough bread (no sourdough). The profiles of volatile compounds in percentage by GC / MS are given in Fig. 1. A total of 54 compounds in breads were determined by comparison with the mass spectra in the GC-MS library. When the sourdough breads made by co-cultivation were compared with the bread made with flat dough (without sourdough), it was observed that the ethanol content was higher. The highest ethanol production was in B and F code breads. In general, it can be said that all three yeast cultures are high alcohol producers. Besides ethanol, 1-hexanol (fruity odor and aromatic flavor), 1-heptanol (fragrant, woody, heavy, oily, faint, aromatic, fatty odor, and a pungent, spicy taste), 1-octanol (fresh, orange-rose odor) Compounds such as 1-nonanol (fresh, orange, rose), phenylethyl alcohol (rose-like odor), and 2-methyl-1-butanol (cooked, roasted aroma with fruity or alcoholic undertones) were also present in breads (Menezes et al., 2018). Another compound commonly produced by yeasts is Phenylethyl alcohol, which releases rose aroma (Longo and Sanromán, 2006). This alcohol was detected in all breads except B and CY. Looking at the results,

Table 2: The result of cell density, pH and TTA values of microorganisms in the I and II phase of sourdough fermentation.

Code of samples	I Phase (log CFU / g)		II Phase (log CFU / g)		I Phase		II Phase	
	Lab	Yeast	Lab	Yeast	pH	TTA (% lactic acid)	pH	TTA (% lactic acid)
A	8.61 ^A	6.81 ^B	8.13 ^B	6.85 ^B	5.34 ^B	0.36	4.96 ^A	0.38
B	8.66 ^A	6.84 ^B	8.32 ^{AB}	6.09 ^C	5.51 ^A	0.43	4.22 ^D	0.81
C	8.61 ^A	8.12 ^A	7.69 ^C	7.62 ^A	4.70 ^C	0.76	4.51 ^B	0.7
D	8.27 ^C	8.05 ^A	8.11 ^B	7.67 ^A	4.25 ^E	0.43	4.24 ^{CD}	0.42
E	8.47 ^B	8.11 ^A	8.37 ^{AB}	7.24 ^{AB}	4.30 ^{DE}	0.68	4.11 ^E	0.78
F	8.61 ^A	7.09 ^B	8.51 ^A	6.20 ^C	4.35 ^D	0.58	4.26 ^C	0.52
CA	-	-	-	-	3.56 ^F	8.78	3.72 ^F	4.05

*Within the column, the letters indicates that there is significant differences between the strains ($p < 0.05$).

A: *Saccharomyces cerevisiae* 06B-39 + *Lpb. plantarum* 45MK-30, B: *Saccharomyces cerevisiae* 06B-39 + *Lim. fermentum* 29GT-19, C: *Kazachstania humilis* KCO-60 + *Lpb. plantarum* 45MK-30, D: *Pichia membranifaciens* 11G-11 + *Lpb. plantarum* 45MK-30, E: *Pichia membranifaciens* 11G-11 + *Lim. fermentum* 29GT-19, F: *Kazachstania humilis* KCO-60 + *Lim. fermentum* 29GT-19, CA (Control): Chemically acidified.

Table 3: Determination of texture profiles of breads.

Bread code	Hardness (N)	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
A	2.37 ^{DE}	0.97 ^A	0.85 ^A	190.03 ^D	179.5 ^C	0.55 ^B
B	2.53 ^{DE}	1.51 ^A	0.87 ^A	219.98 ^{CD}	332.5 ^C	0.57 ^{AB}
C	2.04 ^{DE}	1.39 ^A	0.88 ^A	168.82 ^D	246.6 ^C	0.59 ^{AB}
D	2.68 ^D	1.25 ^A	0.85 ^A	229.54 ^{CD}	290.02 ^C	0.56 ^{AB}
E	4.61 ^C	0.92 ^A	0.85 ^A	394.17 ^C	364.38 ^C	0.55 ^B
F	1.27 ^E	1.31 ^A	0.88 ^A	112.51 ^D	147.35 ^{FC}	0.57 ^{AB}
CA	18.56 ^A	0.86 ^A	0.89 ^A	1653.12 ^A	1419.76 ^A	0.63 ^A
CY	9.54 ^B	0.91 ^A	0.86 ^A	821.55 ^B	754.26 ^B	0.55 ^B

*Within the column, the letters indicates that there is significant differences between the strains ($p < 0.05$).

A: *Saccharomyces cerevisiae* 06B-39 + *Lpb. plantarum* 45MK-30, B: *Saccharomyces cerevisiae* 06B-39 + *Lim. fermentum* 29GT-19, C: *Kazachstania humilis* KCO-60 + *Lpb. plantarum* 45MK-30, D: *Pichia membranifaciens* 11G-11 + *Lpb. plantarum* 45MK-30, E: *Pichia membranifaciens* 11G-11 + *Lim. fermentum* 29GT-19, F: *Kazachstania humilis* KCO-60 + *Lim. fermentum* 29GT-19, CA (Control): Chemically acidified, CY: Flat dough without sourdough.

the acid aroma component of sourdough fermented by *Lpb. plantarum* was relatively high. Since this species is heterofermentative, it produces acid through glycolysis fermentation. More than one volatile compound can be formed in fermentation by yeast and LAB. Studies have shown that ethanol produced by *S. cerevisiae* causes a strong alcoholic aroma in sourdough (Farahmand et al., 2020). Another compound by *S. cerevisiae* is 3-methyl-1-butanol and phenylethyl alcohol. These compounds are formed via the Ehrlich pathway and these are derived from leucine, phenylalanine, respectively (Yang et al., 2020). Another important point in the results was that the compounds l-limonene, isopropyl myristate, 3-p-menthanol, n-decanal, dioctyl phthalate were seen only in A sourdough bread (*S. cerevisiae* + *Lpb. plantarum*). Isopropyl

myristate fatty acid ester has been reported to exhibit high toxicity to major gram-negative microorganisms (Cardoso et al., 2006).

It has been emphasized that the quality and quantity of volatile compounds are affected by some factors. These factors can be physical, chemical and biological. Among them, it has been stated that the physical ones affect the food and oxygen accessibility of microorganisms. Therefore, the rate of formation of volatile metabolites is affected by the sample particle size of the substrate (Parsazad et al., 2020). In addition, it is known that several chemical factors such as carbon and nitrogen level significantly affect the production of aroma during fermentation (Irfan et al., 2016; Farahmand et al., 2021) and it has been stated that microorganisms play an important

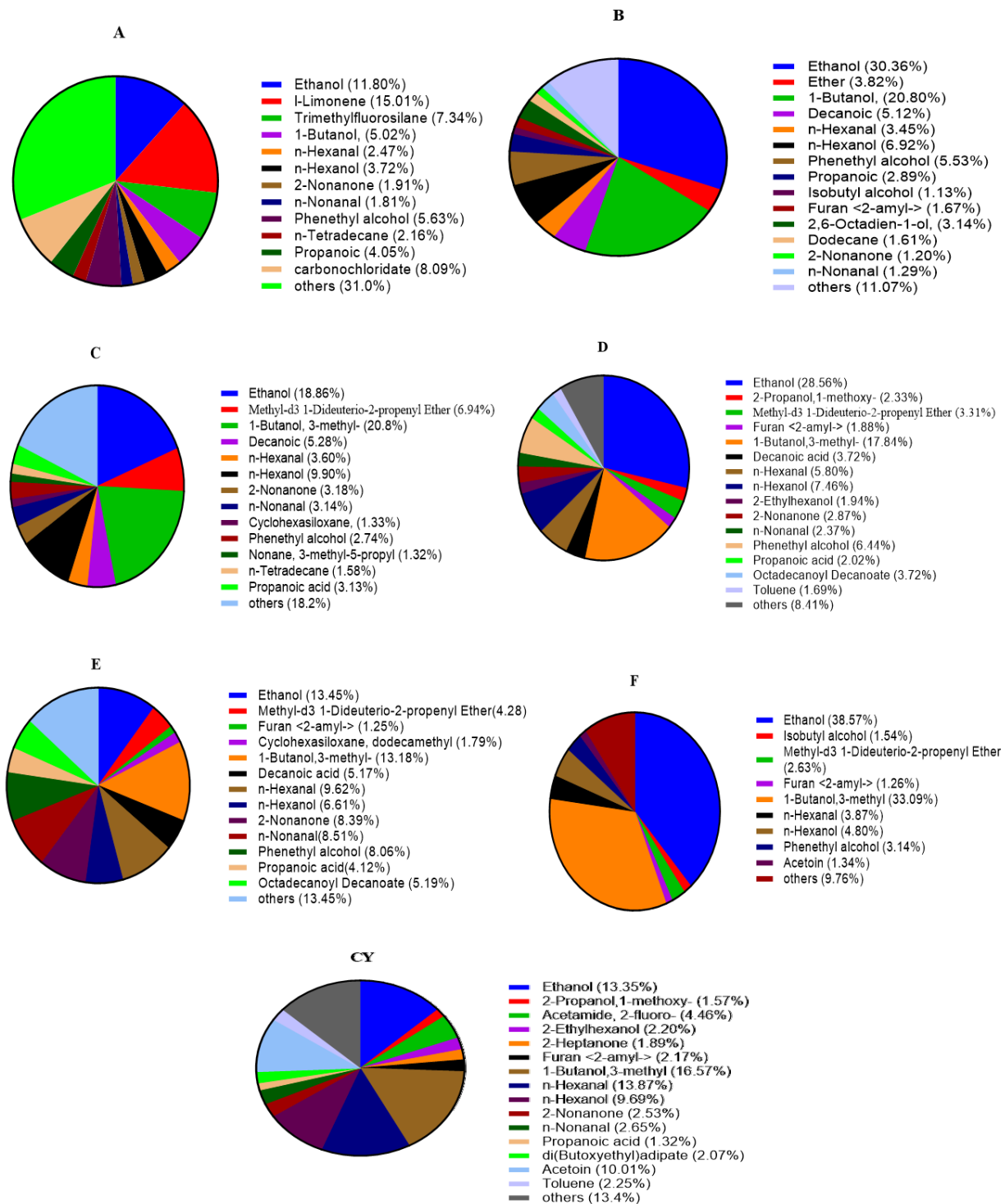


Figure 1: Profile of the volatile compounds in sourdough bread. A: *Saccharomyces cerevisiae* 06B-39 + *Lpb. plantarum* 45MK-30, B: *Saccharomyces cerevisiae* 06B-39 + *Lim. fermentum* 29GT-19, C: *Kazachstania humilis* KCO-60 + *Lpb. plantarum* 45MK-30, D: *Pichia membranifaciens* 11G-11 + *Lpb. plantarum* 45MK-30, E: *Pichia membranifaciens* 11G-11 + *Lim. fermentum* 29GT-19, F: *Kazachstania humilis* KCO-60 + *Lim. fermentum* 29GT-19, G: Flat dough without sourdough.

role in the formation of aromatic precursors that create aroma in food products through microbial or enzymatic reactions (Suo et al., 2020). Also, yeast strains are important in the formation of aroma profiles in bread (Birch et al., 2013). In our study, it was observed that combinations of LAB and yeast in sourdough breads prepared

with selected starters significantly affected the production of volatile compounds and aromatic flavor of the breads. It can be said that these combinations improve the quality of bread compared to bread without sourdough.

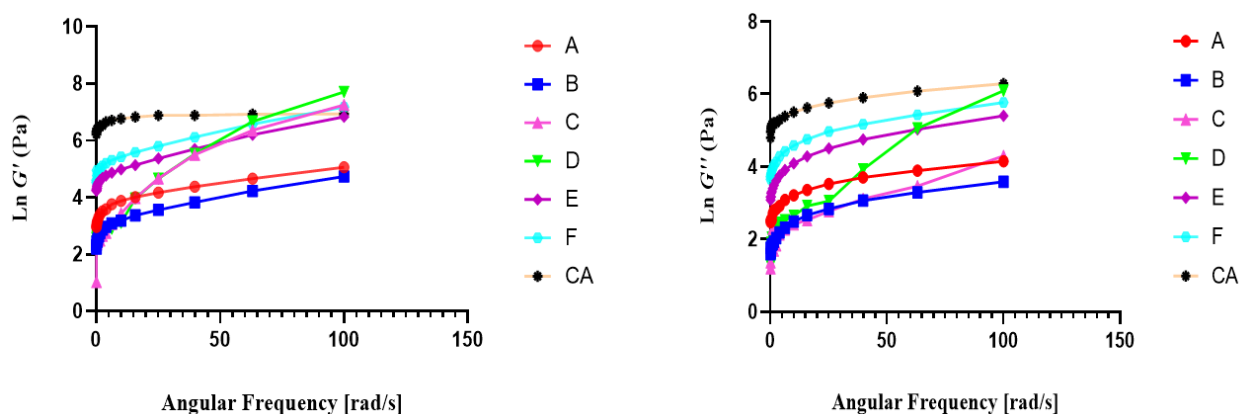


Figure 2: Rheological behavior of dough. A: *Saccharomyces cerevisiae* 06B-39 + *Lpb. plantarum* 45MK-30, B: *Saccharomyces cerevisiae* 06B-39 + *Lim. fermentum* 29GT-19, C: *Kazachstania humilis* KCO-60 + *Lpb. plantarum* 45MK-30, D: *Pichia membranifaciens* 11G-11 + *Lpb. plantarum* 45MK-30, E: *Pichia membranifaciens* 11G-11 + *Lim. fermentum* 29GT-19, F: *Kazachstania humilis* KCO-60 + *Lim. fermentum* 29GT-19, CY: Flat dough without sourdough.

3.4 Viscoelastic property

The rheological properties of wheat doughs containing type II sourdough prepared using different starters after 24 hours of fermentation are given in Fig. 2. While the elastic modulus (G' , storage) determines the elastic property of a material, the loss modulus (G'') determines its viscous property. It was found that G' , G'' values of sourdoughs prepared with co-cultures varied. All of the results were $G' > G''$ and their viscous behavior decreased with fermentation. In contrast, elastic behavior increased. The rheological properties of sourdoughs were evaluated by comparison with the CA (chemically acidified) control dough. Figure 2 shows that elasticity is higher for the control than for the doughs with sourdough.

Many factors can affect the rheology of the dough. At the beginning of the mixing process, the proteins form the gluten network and the starch granule absorbs the water. These affect dough rheology (Wehrle et al., 1997; Voinea, 2020). Because starch granules are reported to be dispersed within the network of gluten proteins. Essentially, it is stated that the gluten network is responsible for the viscoelastic properties, although starch is important (Cappelli et al., 2020). Chemical and physical changes in the gluten network affect the rheological properties. With the progress of fermentation, the protein matrix degrades, making it less flexible (Yildirim-Mavis et al., 2019). Another factor affecting dough rheology is pH. The change in pH as a result of lactic acid production affects dough rheology (Yildirim-Mavis et al., 2019). The decrease in pH values during fermentation causes partial starch degradation. Besides, enzymatic activity increases protease activity and causes weakening of the gluten network (Clarke et al., 2004). On the other hand, it can be said that metabolites accumulate in the dough matrix during the fermentation process and these cause rheological changes. It can be assumed that this rheological behavior is due to the microstructure of the change dough (Alpers et al., 2020). In some studies, it has been said that the effect of yeast on rheological properties is similar to that of H_2O_2 . This effect is mainly due to the production of H_2O_2 by

yeasts. In addition, yeasts make the dough more elastic (Mirsaeedghazi et al., 2008). Besides, CO_2 produced during fermentation lowers the pH by dissolving in water and thus affects the rheology of the fermented dough (Spies, 1990; Mirsaeedghazi et al., 2008). Other compounds produced by yeast (glutathione, glycerol, pyruvic acid) weakens the gluten network. Thus, it contributes to the texture of the bread (Hernández-Parada et al., 2022). Besides, organic acids such as acetic and lactic acid produced by lactobacilli significantly affect the rheological properties of sourdoughs, such as dough gas retention and elasticity of the gluten network (Zaib-Un-Nisa et al., 2016).

Based on the rheological results, it is observed that the modulus values increase with increasing frequency. Rheological measurements were made to determine how each microorganism used in the study affects the viscoelastic properties of the dough. G' was greater than the G'' modulus in all analyzed doughs. The results presented in Fig. 2 show that the sourdough samples are less elastic. This decrease can be attributed to the effect of the pH drop, and the changes that might occur between protein and starch molecules.

4. Conclusions

The parameters of the mixture of LAB and yeast starters on sourdough and sourdough bread were observed. Since, the use of sourdough is important in terms of increasing the shelf life and nutritional value of the bread and improving its organoleptic properties. As a result of the data obtained, the interactions between the yeast and LAB combination caused a change in the sourdough. The use of these sourdoughs in wheat dough caused to exhibit elastic properties of the samples. In addition, it was observed that the breads provided a softer texture and had more aroma compounds and increased the bread baking quality. A strategic combination of *Pichia membranifaciens* 11G-11 + *Lim. fermentum* 29GT-19 could give a desirable fermentation with improved nutrient quality if carried out under controlled conditions. Further studies show that

the combination of these types can be used in the bakery industry. However, it may need to be carried out under controlled conditions. Thus, the potential advantages of sourdough bread can be exploited, because it has shown that LAB and yeast have significant effects on volatile components and rheological properties of breads.

References

- Akinola, S. A., Ateba, C. N., and Osundahunsi, O. F. (2020). Interaction of selected lactic acid bacteria and yeast improves the quality of pearl millet sourdough bread. *J. Microbiol. Biotechnol. food Sci.*, 10(1):22–27.
- Akinola, S. A. and Osundahunsi, O. F. (2017). Lactic acid bacteria and yeast diversities in spontaneously fermented millet sourdoughs. *J. Microbiol. Biotechnol. Food Sci.*, 6(4):1030–1035.
- Alkay, Z., Kilmanoglu, H., and Durak, M. (2020). Prevention of sourdough bread mould spoilage by antifungal lactic acid bacteria fermentation. *Eur. J. Sci. Technol.*, pages 379–388.
- Alkay, Z., Yilmaz, M. T., Can, A. M., İspirli, H., and Dertli, E. (2022). The effect of flours of different immature cereal grains on sourdough and sourdough bread: Microbiological, rheological, textural and sugar profiles. *J. Food Process. Preserv.*, 46(11).
- Alpers, T., Tauscher, V., Steglich, T., Becker, T., and Jekle, M. (2020). The self-enforcing starch-gluten system-strain-dependent effects of yeast metabolites on the polymeric matrix. *Polymers (Basel)*, 13(1):30.
- Arendt, E. K., Ryan, L. A., and Dal Bello, F. (2007). Impact of sourdough on the texture of bread. *Food Microbiol.*, 24(2):165–174.
- Birch, A. N., Petersen, M. A., Arneborg, N., and Hansen, Å. S. (2013). Influence of commercial baker's yeasts on bread aroma profiles. *Food Res. Int.*, 52(1):160–166.
- Cappelli, A., Oliva, N., and Cini, E. (2020). A systematic review of gluten-free dough and bread: Dough rheology, bread characteristics, and improvement strategies. *Appl. Sci.*, 10(18):6559.
- Carbonetto, B., Nidelet, T., Guezenec, S., Perez, M., Segond, D., and Sicard, D. (2020). Interactions between *Kazachstania humilis* yeast species and lactic acid bacteria in sourdough. *Microorganisms*, 8(2):240.
- Clarke, C. I., Schober, T. J., Dockery, P., O'Sullivan, K., and Arendt, E. K. (2004). Wheat sourdough fermentation: Effects of time and acidification on fundamental rheological properties. *Cereal Chem. J.*, 81(3):409–417.
- Corsetti, A., Gobbetti, M., De Marco, B., Balestrieri, F., Paoletti, F., Russi, L., and Rossi, J. (2000). Combined effect of sourdough lactic acid bacteria and additives on bread firmness and staling. *J. Agric. Food Chem.*, 48(7):3044–3051.
- Corsetti, A. and Settanni, L. (2007). Lactobacilli in sourdough fermentation. *Food Res. Int.*, 40(5):539–558.
- De Vuyst, L., Vrancken, G., Ravyts, F., Rimaux, T., and Weckx, S. (2009). Biodiversity, ecological determinants, and metabolic exploitation of sourdough microbiota. *Food Microbiol.*, 26(7):666–675.
- Ewuoso, M. O., Animashaun, O. H., and Adejumo, A. A. (2020). Lactic acid bacteria and yeasts in spontaneously fermented sorghum sourdough. *Am. J. Microbiol. Res.*, 8(2):63–72.
- Farahmand, E., Razavi, S. H., and Mohtasebi, S. S. (2021). Investigating effective variables to produce desirable aroma in sourdough using e-nose and sensory panel. *J. Food Process. Preserv.*, 45(2).
- Fernández-Peláez, J., Paesani, C., and Gómez, M. (2020). Sourdough technology as a tool for the development of healthier grain-based products: An update. *Agronomy*, 10(12):1962.
- Gänzle, M. and Ripari, V. (2016). Composition and function of sourdough microbiota: From ecological theory to bread quality. *Int. J. Food Microbiol.*, 239:19–25.
- Hernández-Parada, N., González-Ríos, O., Suárez-Quiroz, M. L., Hernández-Estrada, Z. J., Figueroa-Hernández, C. Y., Figueroa-Cárdenas, J. d. D., Rayas-Duarte, P., and Figueroa-Espinoza, M. C. (2022). Exploiting the native microorganisms from different food matrices to formulate starter cultures for sourdough bread production. *Microorganisms*, 11(1):109.
- Holzappel, W. (2002). Appropriate starter culture technologies for small-scale fermentation in developing countries. *Int. J. Food Microbiol.*, 75(3):197–212.
- Irfan, M., Asghar, U., Nadeem, M., Nelofer, R., and Syed, Q. (2016). Optimization of process parameters for xylanase production by *Bacillus* sp. in submerged fermentation. *J. Radiat. Res. Appl. Sci.*, 9(2):139–147.
- Kim, Y., Huang, W., Zhu, H., and Rayasduarte, P. (2009). Spontaneous sourdough processing of Chinese Northern-style steamed breads and their volatile compounds. *Food Chem.*, 114(2):685–692.
- Koehler, P. and Wieser, H. (2013). Chemistry of cereal grains. In *Handb. sourdough Biotechnol.* Springer, Boston, MA, USA.
- Longo, M. A. and Sanromán, M. A. (2006). Production of food aroma compounds: Microbial and enzymatic methodologies. *Food Technol. Biotechnol.*, 44(3):335–353.
- Maloney, D. and Foy, J. (2003). Yeast fermentations. In Kulp, K. and Lorenz, K., editors, *Handb. Dough Ferment.* Marcel Dekker, New York, USA.
- Menezes, A. G. T., Ramos, C. L., Dias, D. R., and Schwan, R. F. (2018). Combination of probiotic yeast and lactic acid bacteria as starter culture to produce maize-based beverages. *Food Res. Int.*, 111:187–197.
- Mirsaeedghazi, H., Emam-Djomeh, Z., and Mousavi, S. M. A. (2008). Rheometric measurement of dough rheological characteristics and factors affecting it. *Int. J. Agric. Biol.*, 10(1):112–119.
- Odunfa, S. and Adeyele, S. (1985). Microbiological changes during the traditional production of ogi-baba, a West African fermented sorghum gruel. *J. Cereal Sci.*, 3(2):173–180.
- Olojede, A., Sanni, A., and Banwo, K. (2020). Rheological, textural and nutritional properties of gluten-free sourdough made with functionally important lactic acid bacteria and yeast from Nigerian sorghum. *LWT*,

- 120:108875.
- Oshiro, M., Zendo, T., and Nakayama, J. (2021). Diversity and dynamics of sourdough lactic acid bacteria created by a slow food fermentation system. *J. Biosci. Bioeng.*, 131(4):333–340.
- Parsazad, M., Babaeipour, V., MalekSabet, N., Mohamadian, J., and Masoumian, M. (2020). Optimization of 2,6-dimethoxy benzoquinone production through wheat germ fermentation by *Saccharomyces cerevisiae*. *Appl. Food Biotechnol.*, 7:161–169.
- Poutanen, K., Flander, L., and Katina, K. (2009). Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiol.*, 26(7):693–699.
- Sakandar, H. A., Hussain, R., Kubow, S., Sadiq, F. A., Huang, W., and Imran, M. (2019). Sourdough bread: A contemporary cereal fermented product. *J. Food Process. Preserv.*, 43(3):e13883.
- Sieuwerths, S., de Bok, F. A. M., Hugenholtz, J., and van Hylckama Vlieg, J. E. T. (2008). Unraveling microbial interactions in food fermentations: From classical to genomics approaches. *Appl. Environ. Microbiol.*, 74(16):4997–5007.
- Spies, R. (1990). Application of Rheology in the Bread Industry. In *Dough Rheol. Baked Prod. Texture*, pages 343–361. Springer US, Boston, MA.
- Suo, B., Nie, W., Wang, Y., Ma, J., Xing, X., Huang, Z., Xu, C., Li, Z., and Ai, Z. (2020). Microbial diversity of fermented dough and volatile compounds in steamed bread prepared with traditional Chinese starters. *LWT*, 126:109350.
- Vogel, R., Knorr, R., Müller, M., Steudel, U., Gänzle, M., and Ehrmann, M. (1999). Non-dairy lactic fermentations: The cereal world. *Antonie Van Leeuwenhoek*, 76:403–411.
- Voinea, A. (2020). Effect of dry sourdough addition in wheat flour on dough rheological properties. *Sci. Bull. Ser. F. Biotechnol.*, 24(1):45–49.
- Wehrle, K., Grau, H., and Arendt, E. K. (1997). Effects of lactic acid, acetic acid, and table salt on fundamental rheological properties of wheat dough. *Cereal Chem. J.*, 74(6):739–744.
- Winters, M., Panayotides, D., Bayrak, M., Rémont, G., Viejo, C., Liu, D., Le, B., Liu, Y., Luo, J., Zhang, P., and Howell, K. (2019). Defined co-cultures of yeast and bacteria modify the aroma, crumb and sensory properties of bread. *J. Appl. Microbiol.*, 127(3):778–793.
- Wu, C., Liu, R., Huang, W., Rayas-Duarte, P., Wang, F., and Yao, Y. (2012). Effect of sourdough fermentation on the quality of Chinese Northern-style steamed breads. *J. Cereal Sci.*, 56(2):127–133.
- Xu, D., Zhang, Y., Tang, K., Hu, Y., Xu, X., and Gänzle, M. G. (2019). Effect of mixed cultures of yeast and lactobacilli on the quality of wheat sourdough bread. *Front. Microbiol.*, 10.
- Yang, H., Sadiq, F. A., Liu, T., Zhang, G., and He, G. (2020). Use of physiological and transcriptome analysis to infer the interactions between *Saccharomyces cerevisiae* and *Lactobacillus sanfranciscensis* isolated from Chinese traditional sourdoughs. *LWT*, 126:109268.
- Yildirim-Mavis, C., Yilmaz, M. T., Dertli, E., Arici, M., and Ozmen, D. (2019). Non-linear rheological (LAOS) behavior of sourdough-based dough. *Food Hydrocoll.*, 96:481–492.
- Zaib-Un-Nisa, Salim-Ur-Rehman, Huma, N., and Shahid, M. (2016). Impact of mixed lactic acid bacterial (LAB) culture on flavoring profile and quality attributes of spring wheat sourdough bread. *Pakistan J. Agric. Sci.*, 53(1):225–231.